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Synthesis and secretion of wild-type and mutant human plasma cholesteryl ester transfer protein in baculovirus-transfected insect cells: The carboxyl-terminal region is required for both lipoprotein binding and catalysis of transfer

(lipid metabolism)

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ABSTRACT Functional plasma cholesteryl ester transfer protein (CETP; 476 amino acids) has been expressed in baculovirus-transfected Sf9 insect cells by using a full-length cDNA derived from a human placental library. The product bound to each major plasma lipoprotein class, and it catalyzed the transfer of both cholesteryl esters and triglyceride. CETP species with overlapping deletions were generated in the carboxyl-terminal region. These mutants were defective in cholesteryl ester and triglyceride transfer. Structural and functional analysis suggests that normal lipoprotein binding and effective catalysis may require the carboxyl-terminal sequence -Phe-Leu-Leu-Leu- (residues 454–457), possibly with the involvement of other sequences in the carboxyl-terminal region. A similar sequence is contained in several other proteins whose functions involve binding nonpolar lipids, including lecithin: cholesterol acyltransferase, lipopolysaccharide-binding protein, bactericidal permeability-increasing protein, cholesterol 7 α -hydroxylase, cholesterol esterase, and hormone-sensitive lipase. These data suggest that a conserved neutral lipid-binding sequence may be one important factor in the activity of CETP and possibly in several other proteins of plasma and cellular lipid metabolism.

Human plasma cholesteryl ester transfer protein (CETP) transfers cholesteryl esters and other nonpolar lipids between the major plasma lipoprotein classes (1). Its activity represents an important link in the pathway (reverse cholesterol transport) by which peripheral cell and plasma cholesterol are returned through the plasma to the liver for catabolism (2, 3). This reflects the ability of CETP to transfer cholesteryl esters to lipoprotein acceptors (particularly very low and low density lipoproteins, VLDL and LDL) which are ligands for hepatic lipoprotein receptors (4).

CETP has been purified to homogeneity (5, 6) and its cDNA has been cloned and sequenced (7). This sequence indicates a highly hydrophobic protein with a molecular mass of 53.1 kDa and a polypeptide chain length of 476 amino acids. CETP has a large carbohydrate moiety, and the total molecular mass in plasma has been estimated at 62–74 kDa (5, 6, 8).

The mechanism of CETP has been difficult to determine because of its complex kinetics, the lack of major structural relationships to proteins of known tertiary structure, and the presence of hydrophobic sequences throughout the molecule that could represent potential lipid-binding sites. Most investigators have found that plasma CETP forms complexes with all the major plasma lipoprotein classes (9, 10), and it is likely that the formation of such a complex between two different

lipoprotein particles is the initial step in successful lipid transfer. CETP catalyzes both the heteroexchange of cholesteryl ester for triglyceride and the conservative exchange of cholesteryl esters or triglycerides.

Baculovirus-driven expression systems provide a powerful technology to express many wild-type and mutant N-glycosylated proteins, since lepidopteran (Sf9) cells synthesize most of the enzymes required for the initial linkage and early processing of mammalian protein-linked carbohydrate chains. In Sf9 cells, N-linked oligosaccharides can be processed to produce oligomannose side chains (11–13). In the present research we have used a nuclear polyhedrosis virus to drive the expression and secretion of active human plasma CETP. Site-directed mutagenesis was then used to investigate the structural requirements for lipoprotein binding and transfer.

EXPERIMENTAL METHODS

Plasmid Construction. A full-length cDNA for human CETP was isolated from a human placental cDNA library in λ gt11 (the gift of Pat Stanislavitis, Massachusetts General Hospital, Boston). Its entire sequence was obtained by the dideoxy method (14). The 1.7-kilobase (kb) cDNA contained 13 nucleotides of 5' untranslated region, the entire coding region, and 178 nucleotides in the 3' untranslated region. One sequence difference was found in comparison with the cDNA sequence published earlier (7). The substitution A \rightarrow G was found at nucleotide +1394, resulting in a conservative change of isoleucine to valine in the translated protein sequence.

Recombinant baculoviral vectors containing CETP cDNA were constructed by using the vectors pVL1392 and pVL1393 (ref. 15; generously provided by M. Summers, Texas A & M University, College Station). The cDNA was inserted into the unique *Eco*RI site of pVL1393, and a unique *Xho* I site in pVL1393 was used to determine orientation of the CETP cDNA in the transfer vector (15). The expression of nonfused CETP in pVL1393 requires the translation initiation codon (ATG) from the CETP cDNA and contains the consensus sequence for polyadenylation from the CETP cDNA as well (Fig. 1).

CETP cDNA species with deletions in the 3' region of coded sequence were constructed to generate nested deletions including the 26-amino acid carboxyl-terminal fragment. The carboxyl-terminal amino acid sequences represented by these constructions are shown in Table 1. The plasmid pCETP-66 codes for the CETP cDNA with amino

Abbreviations: CETP, cholesteryl ester transfer protein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; AcMNPV, *Autographa californica* nuclear polyhedrosis virus.

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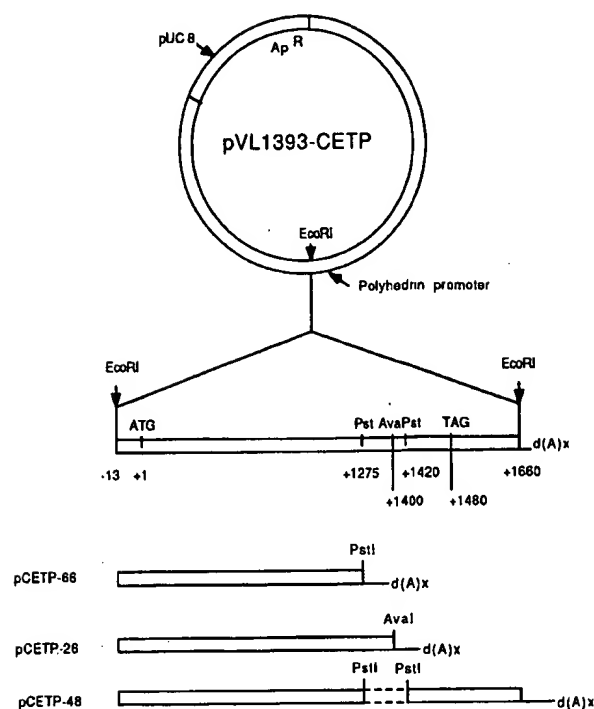


FIG. 1. Structure of recombinant CETP baculovirus transfer vectors. pVL1393-CETP contains the full-length CETP cDNA inserted at the unique *EcoRI* site of pVL1393. The first nucleotide from the coding region (ATG) is numbered +1. Plasmids pCETP-66, pCETP-26, and pCETP-48 have undergone deletions of sequences encoding 66, 26, and 48 amino acid residues, respectively, at the carboxyl terminus. *PstI* and *AvaI* are restriction endonuclease sites used for the deletion constructions.

acids 411–476 deleted. It was constructed by digesting the wild-type CETP cDNA with *PstI* and *EcoRI*. The 1.3-kb *PstI*/*EcoRI* fragment was inserted into pVL1393 that had been digested with the same restriction endonucleases.

The plasmid pCETP-48 codes for CETP with amino acids 411–458 deleted (Fig. 1). It was constructed by digesting wild-type CETP cDNA with *PstI*. The 144-base-pair (bp) *PstI* fragment was deleted, allowing the religation of the remaining 1.6-kb CETP cDNA.

The plasmid pCETP-26 was prepared by a two-step procedure. Wild-type CETP cDNA was first digested with *AvaI*, then blunt-ended with T4 DNA polymerase. A 1.4-kb CETP cDNA fragment was then produced by *EcoRI* digestion. The *EcoRI*/blunt-ended DNA was inserted into pVL1392 that had been digested with *EcoRI*/*SmaI*.

Cells and Viruses. Sf9 cells of the fall armyworm, *Spodoptera frugiperda* (American Type Culture Collection CRL 1711), were maintained as suspension cultures in TN-MFH medium (16) containing 10% heat-inactivated fetal bovine serum and gentamycin at 50 μ g/ml. The cells were subcultured every 2–3 days from $3.5\text{--}4 \times 10^6$ down to 1×10^6

cells per ml. The E2 strain of wild-type *Autographa californica* nuclear polyhedrosis virus (AcMNPV) and the recombinant baculovirus encoding human CETP (bhCETP) were propagated and titrated in Sf9 cells as previously described (16). For the preparation of bhCETP baculovirus, the Sf9 cells were transfected with 2 μ g of transfer vector containing CETP cDNA and 1 μ g of viral DNA. After three rounds of plaque purification, the recombinants were amplified in two rounds by infecting monolayers of cells for 3 days. The supernatant was then clarified by low-speed centrifugation. When viral titers were 10^7 to 10^8 plaque-forming units/ml, recombinants were assayed for expression of CETP activity at a multiplicity of infection of 2.5 at 28°C, in Sf9 cells adapted to serum-free medium (Excell 400, JRH Biosciences, Woodland, CA) containing gentamycin. The time course of CETP secretion by recombinant CETP baculoviruses was monitored by dot blot immunoassay (see below). Culture supernatants were measured for CETP mass at days 1, 2, and 3 after infection, using multiplicities of infection of 2.5, 10, and 50.

Determination of CETP Synthesis and Secretion. After infection as described (16), the culture medium was assayed for secreted CETP, using a rabbit antibody raised against residues 290–306 of the mature plasma protein (7, 10). Assays were carried out with a 96-well ultrafiltration unit (Minifold, Schleicher & Schuell) with nitrocellulose membrane (Sartorius) as solid phase. After application of up to 100 μ l of medium, the sheet was incubated for 24 hr at room temperature with affinity-purified CETP antiserum. The membrane was then washed (three times) for 30 min with 2% bovine serum albumin/0.2% Nonidet P-40/0.01 M Tris-HCl/0.15 M NaCl, pH 7.4, then finally with goat anti-rabbit IgG (Boehringer Mannheim) labeled with 125 I for 30 min at room temperature. After washing (three times) with Tris/saline, radioactivity in individual samples and in standards of isolated CETP (5) was measured by γ -ray spectrometry. The signal obtained was linear over the range 2–50 ng of CETP.

Assay of Cholesteryl Ester Transfer Activity. CETP was assayed as the rate of transfer of [3 H]cholesteryl linoleate (5) from human plasma LDL to human or rat high density lipoprotein (HDL). (There was no difference in the rate of transfer under these conditions, but the endogenous transfer activity was low or absent from rat HDL.) LDL was isolated by ultrafiltration within the density range 1.02–1.05 g/ml in the presence of 1 mM disodium EDTA, pH 7.4. HDL was isolated within the density limits 1.08–1.21 g/ml under the same conditions. Lipoprotein cholesteryl ester in HDL and LDL was measured enzymatically by an autoanalyzer as the difference between total and free cholesterol. Lipoprotein protein was determined with serum albumin as standard. LDL cholesteryl ester was labeled as previously described (5) with [1,2- 3 H]cholesteryl linoleate (New England Nuclear) to a final specific activity of $1\text{--}2 \times 10^3$ cpm/ μ g.

Assays contained [3 H]cholesteryl ester-labeled LDL (300 μ g of cholesteryl ester), unlabeled HDL (50 μ g of cholesteryl ester), recrystallized human serum albumin (final concentration 10 mg/ml), and 0.025 M sodium phosphate buffer, pH 7.5, in a final incubation volume of 0.5 ml. These conditions were previously demonstrated to maximize transfer rates in this system (5). Assay mixtures also contained a final concentration of 1.4 mM dithiothreitol (2-nitrobenzoic acid), to inhibit any residual lecithin:cholesterol acyltransferase (17), whose activity could dilute the labeled cholesteryl ester pool during assay. Incubation was for up to 5 hr at 37°C, during which transfer of cholesteryl ester to HDL was linear (5). LDL was precipitated on ice for 10 min with dextran/MgCl₂ (final concentrations 5 mg/ml and 0.01 M, respectively) (18). The precipitate of LDL was then removed by centrifugation (0°C, 2000 \times g, 20 min). Cholesteryl ester radioactivity in the

Table 1. Overlapping deletions in human CETP

434	NSKGVSLFDIINPEIITRDGFLLLQMDFGPPEHLLVDFLQSL	476	WT
	NSKGVSLFDIINPEIIT		CETP-26
		QMDFGPPEHLLVDFLQSL	CETP-48
			CETP-66

Amino acid residues that have been deleted from the wild type (WT) are shown by the hyphens. The carboxyl-terminal 26 amino acids have been deleted from CETP-26, while the carboxyl-terminal 66 residues are missing from CETP-66 (residues 410–476). CETP-48 has undergone an internal deletion of residues 410–457. All three mutants are missing the underlined sequence, RDGFLLL.

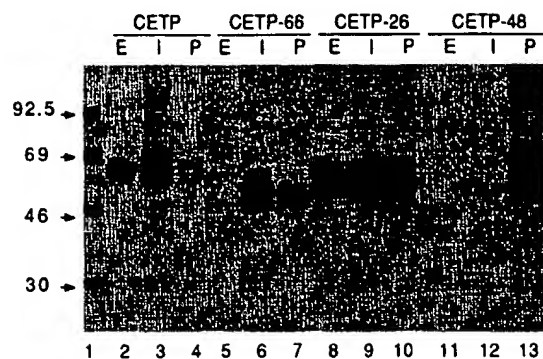


Fig. 2. Immunoblots of wild-type and deletion mutant polypeptides. Cells were infected with recombinant CETP baculoviruses and harvested 3 days after infection. Protein in the extracellular medium (E) was concentrated before SDS/10% polyacrylamide gel electrophoresis and transfer to nitrocellulose and incubation with rabbit antibody to CETP and 125 I-labeled goat antibody to rabbit IgG. Intracellular (I) and pellet (P) fractions were produced by cell lysis with 1% Nonidet P-40 (19). Lane 1, 14 C-methylated protein standards (in kDa); lanes 2–4, wild-type CETP; lanes 5–7, CETP-66; lanes 8–10, CETP-26; lanes 11–13, CETP-48.

supernatant HDL fraction was then determined by liquid scintillation spectrometry.

Determination of CETP Binding to Plasma Lipoproteins. VLDL was isolated by flotation at density < 1.019 g/ml, and LDL and HDL, as described above. VLDL, LDL, or HDL (26 mg of protein) was coupled to CNBr-activated Sepharose 6B (Pharmacia) by suspending the lipoproteins in 0.1 M NaHCO_3 , pH 8.3, containing 0.5 M NaCl with the activated Sepharose. After coupling, CNBr-Sepharose reactive groups were blocked by incubation with 0.1 M Tris-HCl, pH 8. The coupling efficiencies were 95%, 88%, and 71% for LDL, HDL, and VLDL, respectively. Sepharose-bound lipoproteins were washed with 1 M NaCl, then resuspended in 10 mM Tris-HCl, pH 7.4/150 mM NaCl/1.5 mM EDTA (50%, vol/vol). For control experiments, activated Sepharose was incubated only with 0.1 M NaHCO_3 /0.5 M NaCl.

Medium from cells transfected with the human CETP gene was incubated with Sepharose-bound lipoproteins or Sepharose alone for 2 hr at 37°C with continuous stirring in flat-bottomed glass vials. The mixtures were then centrifuged and the supernatants were collected for immunoassay. CETP bound with Sepharose-bound lipoproteins was compared with CETP bound to Sepharose alone. The percent bound was calculated as the free CETP mass in suspensions incubated with Sepharose alone minus that recovered from suspensions incubated with Sepharose-bound lipoprotein, divided by the amount of free CETP mass incubated with Sepharose alone.

RESULTS

Secretion and Properties of Recombinant Wild-Type CETP. Assay of culture medium after the infection of Sf9 cells with

the vector pVL1393-CETP (Fig. 2) demonstrated the appearance of protein reactive with anti-CETP antibody that was absent from mock-transfected or AcMNPV-infected cells. Regardless of the multiplicity of infection, the concentration of CETP protein in the medium reached a maximum at 2 days after infection. As shown in Table 2, specific activity secreted into the medium for the transfer of cholesteryl ester from LDL to HDL was 9.6 ± 0.7 μg of cholesteryl ester transferred per hr per μg of CETP (equivalent to 1.3 ± 0.1 $\mu\text{g}/\text{ml}\cdot\text{hr}$ at a CETP mass of 135 ng/ml). This rate is comparable to the rate of 4.5–11.4 μg of cholesteryl ester per hr per μg of CETP reported for the human plasma protein (5, 6).

SDS/gel electrophoresis indicated that the apparent molecular mass of wild-type CETP secreted under these conditions was about 60 kDa, which may be compared with 72–74 kDa for the plasma protein under the same conditions (Fig. 2, lane 2). As also shown in Fig. 2, lanes 3 and 4, cell extracts contained immunoreactive material with the same properties, together with a smaller species of about 55 kDa. This is comparable to the protein molecular mass (53.1 kDa) of CETP. In the presence of tunicamycin, which blocks the attachment of N-linked high-mannose chains, only the 55-kDa species was present in the cell extract, with little or no immunoreactive protein in the medium. Mock-infected or AcMNPV-infected cells produced no detectable immunoreactive material.

These data suggest that glycosylation is required for the effective secretion of CETP and that nonglycosylated CETP is synthesized but retained within the cells. Sf9 cells synthesize oligomannose but no complex N-linked carbohydrate chains (11–13). This also indicates that the CETP species containing only oligomannose chains is catalytically active.

Secretion and Properties of CETP Mutants With Carboxyl-Terminal Deletions. When the Sf9 cells were infected with baculovirus constructions coding for products with deletions of 66, 48, and 26 amino acids (Table 1), products with the predicted reduced molecular mass were obtained. As shown in Fig. 2, the pCETP-26 construct secreted two truncated CETP species with apparent molecular masses of 60 and 56 kDa. Construct pCETP-48 yielded an intracellular product with an apparent molecular mass of 53 kDa, while construct pCETP-66 secreted a product of about 49 kDa. The region deleted contains none of the N-glycosylation sites of the mature CETP sequence.

The mass of CETP secreted by cells transfected with the truncated cDNA species was in all cases similar to that recovered from transfections with wild-type cDNA. However, transfer activity was undetectable for the mutant proteins (Table 2). These data indicate that each of the three deletions includes a sequence that is absolutely required for effective transfer of cholesteryl esters.

Mutant CETP species were also completely unable to catalyze the transfer of triglyceride between lipoproteins (Table 2). This provides further evidence for the identity of cholesteryl ester and triglyceride transfer sites in CETP.

Lipoprotein Binding by Wild-Type and Mutant CETP Species. CETP secreted into the culture medium of cells infected

Table 2. Cholesteryl ester and triglyceride exchange activity and immunoreactive CETP mass in Sf9 cells infected with wild-type and mutant CETP baculoviruses

Baculovirus	Cholesteryl exchange, $\mu\text{g}/\text{ml}\cdot\text{hr}$	Triglyceride exchange, $\text{ng}/\text{ml}\cdot\text{hr}$	CETP mass, ng/ml
Wild-type CETP	1.3 ± 0.1	0.026 ± 0.003	135 ± 11
CETP-66	0	0.011 ± 0.004	65 ± 8
CETP-26	0	0	96 ± 17
CETP-48	0	0.012 ± 0.004	81 ± 9
Mock infection	0.09 ± 0.16	0.014 ± 0.003	0

Values reflect transfer of cholesteryl ester or triglyceride from LDL (300 μg of cholesteryl ester) to HDL (50 μg of cholesteryl ester) and are mean \pm SD.

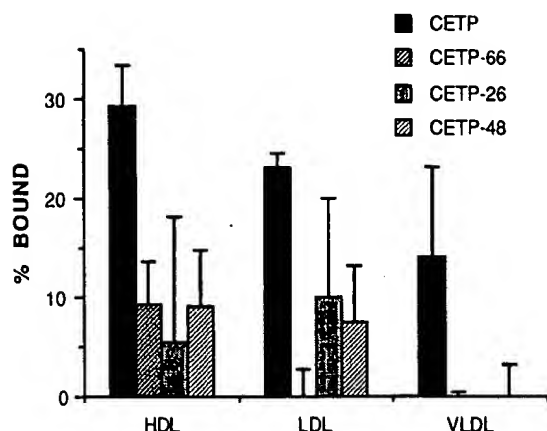


FIG. 3. Binding of CETP proteins to Sepharose-bound lipoproteins. VLDL- (0.02 nmol), LDL- (0.32 nmol), and HDL-Sepharose (3.7 nmol) were resuspended with culture supernatants from wild-type and mutant CETP baculoviruses and incubated for 3 hr at 37°C. Percent of CETP bound to lipoprotein was determined relative to the amount of CETP bound to Sepharose alone.

with wild-type or mutant cDNA species was assayed for its ability to bind to plasma lipoprotein-agarose complexes. Wild-type CETP showed concentration-dependent binding to VLDL, LDL, and HDL. Maximal binding was greatest to HDL, but significant binding was obtained to each lipoprotein. Half-maximal binding to HDL was obtained at 10 ng of CETP (Fig. 3). When the same studies were carried out with the mutant CETP species containing deletions of 26, 48, and 66 amino acids of carboxyl-terminal sequence, significantly lower affinity was obtained, although detectable residual binding was found in each case. These results indicate that the carboxyl-terminal moiety of CETP plays a major role in the binding of the protein to lipoproteins.

DISCUSSION

Sf9 insect cells infected with a baculovirus-derived vector that contained a full-length human CETP cDNA synthesized and secreted functionally active CETP with a specific activity similar to that of the plasma protein. Highly glycosylated proteins such as CETP are often poorly secreted from transfected mammalian cell lines, possibly because of a requirement for complex carbohydrate signals for secretion (20) or because glycosylating enzymes in the endoplasmic reticulum or Golgi complex become rate limiting when expression of the glycoprotein is driven by a strong promoter (21). Sf9 cells lack the enzymes necessary to convert N-linked oligomannose chains into complex carbohydrates. Nevertheless these cells secrete a functional CETP protein; this secretion is inhibited when tunicamycin prevents the formation of N-linked oligomannose chains (22). The active CETP secreted has a smaller molecular weight than the plasma species. This indicates, first, that unmodified high-mannose chains on CETP provide a sufficient signal for effective secretion; and second, that while these chains are secondarily modified in the plasma protein, these modifications do not have much effect on specific activity.

Although the wild-type CETP construction secretes only the glycosylated species, the mutants secrete the nonglycosylated forms also. This may indicate that the carboxyl-terminal region of CETP is important for proper secretion and is involved in proper folding of the polypeptide.

This study extends previous data obtained with a monoclonal antibody binding to the carboxyl-terminal 26-amino acid epitope, which inhibited plasma CETP activity (23). Deletion of the carboxyl-terminal 26 amino acids of the mature CETP abolished the ability of this protein to transfer either cho-

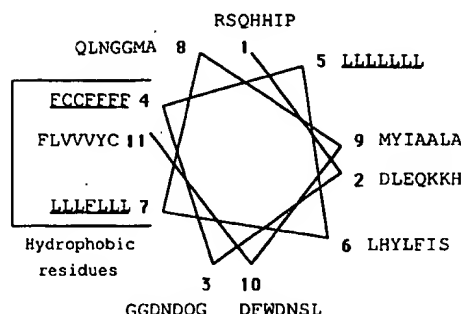


FIG. 4. Helical wheel analysis (25) of the consensus amino acid sequences from CETP, human lecithin:cholesterol acyltransferase, human cholesterol esterase, human bactericidal permeability increasing protein, human lipopolysaccharide binding protein, human cholesterol 7 α -hydroxylase, and rat hormone-sensitive lipase (amino acids at each position are listed from left to right, in the order in which these proteins are listed) (see Table 3). Residues that are conserved in five or more proteins are underlined. The hydrophobic residues are enriched on one side of the helix (in the three-sided box).

lesteryl ester or triglyceride. Analysis of the effect of nested deletions including this region allows a necessary sequence to be further localized to the region spanning residues 454–457 (Table 1). This highly hydrophobic sequence is conserved in human bacterial lipopolysaccharide binding protein (LBP), which shows a 23% overall sequence similarity to CETP (24). This is not the case with neighboring sequences such as PEITRD (residues 446–453) and QMDFGFPEH (residues 458–466) which have a similar overall hydrophobic index, but are apparently absent from LBP (7, 24): Together these data suggest that the neutral lipid-binding site involves the hydrophobic sequence within the larger 26-amino acid carboxyl-terminal sequence. Secondary structure predictions of this region indicate that an octapeptide forms an α -helix (Fig. 4).

While the data in this study point to a role for the hydrophobic helical sequence at position 454–457, the contribution of other regions outside the carboxyl-terminal 66 amino acids is not ruled out, nor is the possibility that two different regions of the protein might act sequentially to effect lipid transfer. An analysis of the three-dimensional structure of CETP will be required to determine this. Nevertheless, the present studies appear to identify one local and important element for the binding reaction of cholesteryl ester and triglyceride.

Information was also obtained on the mechanism by which CETP-mediated catalysis promotes the exchange of cholesterol esters. CETP was found to bind strongly and to a similar extent to the major plasma lipoprotein classes (HDL, LDL, and VLDL) when these were covalently complexed to agarose. There has been disagreement concerning the specificity of CETP binding to lipoproteins, one laboratory finding significant association only with HDL (26) and others reporting that CETP formed stable associations with all the major lipoprotein classes (9, 10). The data in this research support the latter view. Binding was greatest to HDL but significant to both VLDL and LDL. Binding of CETP was significantly reduced in all cases when carboxyl-terminal deletions were made in the structure of CETP. The inhibition of binding was most marked in the case of VLDL, but on average about 75% of binding was lost in the binding of CETP to HDL and LDL. This is the opposite effect to that described when a monoclonal antibody was directed against the carboxyl terminus (23), when inhibition of transfer activity was associated with an anomalous increased binding of CETP to lipoproteins, particularly VLDL. The mechanism of this is not clear, although it may represent a secondary effect of the IgG Fab fragment. The present studies suggest, on the contrary, that the carboxyl-terminal protein element involved in transfer is

Table 3. Similarity between hydrophobic sequences in lipid-binding proteins

Protein	Fragment	Residues	Ref.
Human CETP	-FLLQMDP-	454-461	7
Human LCAT	-CLHLLYFL-	184-191	29
Human CEase	-CLYLNIVV-	80-87	30
Human BPI	-FLLFGADV-	446-453	31
Human LBP	-FLFLGANV-	440-447	24
Human CYP7	-FLILMSY-	457-464	32
Rat lipase	-FLSLAALC-	734-741	33
Consensus	-FLXLXXXn-		
	C		

Amino acid sequences of similar regions from human CETP, human lecithin:cholesterol acyltransferase (LCAT), human cholesterol esterase (CEase), human bactericidal permeability increasing protein (BPI), human lipopolysaccharide binding protein (LBP), human cholesterol 7 α -hydroxylase (CYP7), and rat hormone-sensitive lipase. The consensus sequence for the octapeptide contains a phenylalanine or a cysteine residue at position 1, leucine residues at positions 2 and 4, and a nonpolar residue (n) at position 8. The sequence occurs in the carboxyl-terminal region for all proteins except for LCAT and CEase.

either the same as or overlaps substantially in primary sequence with residues responsible for the major part of CETP binding to lipoproteins.

Two theories have been proposed to explain the kinetics of cholesteryl ester transfer catalyzed by CETP. In the first, the transfer protein is envisaged to act as a shuttle, in which lipoprotein-free CETP containing bound neutral lipid acts as a soluble carrier of these lipids between lipoproteins, effectively increasing the negligible aqueous solubility of triglyceride and cholesteryl ester (27). In the other, CETP was proposed to form a transient ternary complex between donor and acceptor particles (28). The present findings that CETP binds to all major lipoprotein classes and that the regions promoting lipoprotein binding and lipid transfer appear to be the same both support the latter hypothesis.

Finally, the structural motif proposed here as the neutral lipid-binding site in human CETP has counterparts in several other proteins whose functions indicate likely requirements for the binding of unimolecular neutral lipid. As shown in Table 3, there is a considerable sequence similarity between octapeptides in CETP (7, 34), lecithin:cholesterol acyltransferase (LCAT) (29, 35, 36), pancreatic cholesterol esterase (30), bactericidal permeability increasing protein (33), lipopolysaccharide binding protein (24), cholesterol 7 α -hydroxylase (32), and hormone-sensitive lipase (33). The similarity is also marked in the corresponding nucleotide sequences. The functions of all these proteins are likely to require the binding of molecular nonpolar lipid. In all of these proteins, an α -helix is predicted from secondary structure analysis. The α -helix is present within the carboxyl-terminal region of each protein, except for LCAT and cholesterol esterase. This structure may therefore represent the binding site for nonpolar lipid in several otherwise unrelated proteins where this is a substrate (as in cholesterol esterase, cholesterol 7 α -hydroxylase, or hormone-sensitive lipase), a product (LCAT), or a ligand (as in CETP, lipopolysaccharide binding protein, and bactericidal permeability increasing protein). It should now be possible to use site-directed mutagenesis to define the minimal structural features required for effective cholesteryl ester and triglyceride binding.

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